

CME Article

Hiv Resistance Testing

A Clinical Tool

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Drug resistance threatens to erase the recent gains made in treating HIV infection. HIV resistance testing offers clinicians the ability to screen patients for resistant strains and adjust treatment accordingly. Resistance testing is recommended for patients on antiretroviral agents with virologic failure or with suboptimal suppression of viral load after initiation of antiretroviral therapy. Because of the potential transmission of resistant strains, testing should be considered for patients with acute infection. Although the care of many patients with HIV disease is complex and should be managed by a physician with experience and expertise in HIV disease, all practitioners should be familiar with the types of testing available and their limitations.

LEARNING OBJECTIVES

- I. To learn two clinical presentations for which resistance testing is recommended.
- II. To recognize the difference between genotypic and phenotypic resistance tests.
- III. To learn the limitations of resistance testing.

The advent of highly active antiretroviral therapy brought hope to a field in which there was previously very little. The development of new classes of drugs, the refinement of older classes, and an improved understanding of pharmacokinetics and adherence issues have all combined to enhance both the life span and the quality of life for many individuals infected with human immunodeficiency virus (HIV). However, all the progress that has been made with pharmacology can easily be erased by a single naturally occurring phenomenon: drug resistance.

Resistance is hardly a new issue in the manage-

ment of infectious diseases. Practically since the onset of the antibiotic era, clinicians have worked to meet the challenge of eradicating infections in the face of rapidly developing drug resistance among pathogens. The precise incidence of drug resistance among patients with HIV, whether in New Jersey or the nation as a whole, cannot be estimated, because population-based studies have not yet been performed. However, several small studies conducted recently have identified an alarming prevalence of drug resistance among treatment-naïve HIV-positive patients; in one analysis, prevalence was as high as 26%.^{1,2,3,4} As expressed in an editorial in *Hiv Newslines*, “the prevalence of this disturbing phenomenon (HIV drug resistance) is plainly on the increase.”⁵ Resistance has been documented to develop against any one of the currently approved HIV antiretroviral agents. Strains resistant to multiple drugs have also been identified.

By understanding and anticipating resistance

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DISCLOSURE STATEMENT: Sindy M. Paul, MD, MPH, has no relationships to disclose. Victor S. B. Jorden, MD, MPH, is an employee of Abbott Laboratories, which manufactures antiretroviral drugs and HIV testing materials. The article may discuss an unapproved or off-label use of products.

patterns for a given set of circumstances, physicians have often successfully avoided the administration of useless drugs. However, a more scientific and effective approach has been to define a pathogen's drug susceptibilities for each individual patient. Unfortunately for patients infected with HIV, resistance testing has not been available until now. As a result, HIV-infected patients with treatment failures attributed to drug resistance have classically been treated empirically, usually by altering the entire drug regimen.⁶ This drastic measure has been necessary even if the virus is resistant to only one drug of the regimen, since identifying the useless drug has been impossible.

Two recent prospective investigations support the role of resistance testing in clinical practice. The European Viradapt study as well as the U.S. Genotypic Antiretroviral Resistance Testing (GART) study compared the virologic response to antiretroviral therapy with and without the availability of genotypic resistance testing. The results of both studies demonstrated that guiding antiretroviral therapy with resistance testing resulted in a significantly lower viral load, as compared with clinical judgment alone.^{7,8} Similar findings of the advantage of resistance testing using a phenotypic assay versus clinical judgment alone were found in a prospective multicenter study.⁹ These benefits were conferred despite the difficulties and inconsistencies in the interpretation of resistance assays.

Several small studies conducted recently have identified an alarming prevalence of drug resistance among treatment-naïve HIV-positive patients. Clearly, a new era for treatment of HIV has arrived. As a result of recent technological refinements and supporting evidence from clinical trials, resistance testing for HIV is now a clinical reality. Furthermore, reliable resistance testing will tremendously facilitate research into the epidemiology of the transmission of highly resistant strains, an alarming and perhaps growing component of the AIDS epidemic.¹⁰ This article reviews the basic biology of HIV-drug resistance, the techniques used to identify resistance, and the current utility of these techniques.

BASIC BIOLOGY OF RESISTANCE

Resistance may be defined as the natural ability of a microorganism to withstand the effects of a drug that are lethal to most members of its species.¹¹ Host and pharmacological factors, such as persistently subtherapeutic levels of a drug, may play a role in the evolution of HIV resistance to antiretroviral agents. However, the most significant contributors to the development of resistance are those factors related to the virus itself.

HIV is a retrovirus with a profound ability to reproduce its genetic material, a process called replication. An active HIV infection in a typical patient may produce as many as 10 billion new virions in a 24-hour period.¹² However, not every replicated virion will have a genetic pattern identical to its precursor, because the reverse transcriptase gene responsible for the replication rate, combined with the relatively low fidelity of reverse transcriptase in making genetically correct copies, results in practically every possible mutation of the HIV genome on a daily basis.¹³ If the mutation occurs on a part of the gene that codes for an enzyme targeted by antiretroviral medication (i.e., a critical protease), the abnormal protease enzyme that results from the mutation may have a new structure or conformation that makes it resistant to protease inhibitors.

Not only may multiple resistant mutations be formed on a daily basis, but several mutant strains may also coexist in the same patient. HIV infections are polymorphic: every patient's viral profile consists of several viral strains, reproducing at various rates in a given patient at the same time. The multiple strains may be transmitted to the patient at the original time of infection; these are referred to as primary mutations. Alternatively, resistant mutant strains may develop as a result of faulty replication after the original infection; these mutations are referred to as secondary mutations. Both scenarios may contribute to treatment failure.

Not every mutant strain, whether primary or secondary, will survive. Many mutations will lack the necessary biologic characteristics to subsist,

while other strains will have growth and survival capabilities exceeding that of the average strain.¹⁴ The predominant, fast growing strain is called the wild-type strain. However, even if one wild-type strain becomes the principal virus found in the body, other strains may still circulate in very low and virtually unmeasurable concentrations. Furthermore, resistant strains may “hibernate” in lymphoid tissue or other reservoirs within the body. Should the wild-type moiety be successfully attacked by antiretroviral agents, the previously obscure drug-resistant strain may quickly multiply to become the predominant strain, since it is not susceptible to the effects of the therapy with which the patient has been treated. This phenomenon is known as emergence under selective drug pressure.

RESISTANCE TESTING TECHNIQUES

The goal of resistance testing is to identify which drugs will be helpful in controlling HIV infection. The two types of resistance testing are genotypic, in which the patient’s HIV genome is defined, and phenotypic, in which the actual HIV virus from the patient is subjected to varying medications and its growth characteristics studied.

Phenotypic and genotypic assays have been valuable in evaluating antiretroviral agents in clinical trials. Retrospective studies using phenotypic and genotypic assays have shown a correlation between initial drug resistance and virologic outcome of the next regimen.¹⁵ Prospective studies including GART and VIRADAPT found that antiretroviral regimen selection based on genotypic assays had better HIV suppression compared with empirical treatment.^{7, 8}

GENOTYPIC TESTING

Genotypic testing uses sample HIV from the patient and, through polymerase chain reaction or other technologies, examines for the presence of mutations on various parts of the genome. The analysis is generally confined to the specific areas of the gene

(codons) representing proteins where antiretroviral medications are known to exert their action, such as the various proteases. However, some assays involve sequencing the entire reverse transcriptase and protease genes. The patient’s genetic material is compared with known mutation patterns that have been recognized as correlating with specific patterns of resistance. Thus, if an individual patient’s HIV genome has a pattern associated with resistance to a specific antiretroviral agent, that drug may be purposely avoided in therapy. Genotypic assays can be performed relatively rapidly, such that results can be reported within one or two weeks of sample collection.¹⁶

The most serious problem associated with genotypic testing is interpretation. The genomes of measurable HIV strains are first defined and then compared with known genomic templates that are related to drug resistance. Unfortunately, the resistance patterns associated with all possible single mutations have not been identified, which is not surprising considering that the HIV genome contains 9,200 nucleotides. In addition, multiple mutations may appear on a single genome, complicating interpretation of known resistance patterns, since a mutation in one part of the genome may suppress or even obviate the effect of a mutation in another part of the genome.^{17,18} Finally, a recent study examining the interpretation of standardized HIV genomes in different laboratories revealed significant interlaboratory differences, indicating that even for known mutation-induced resistance patterns, not all experts are in agreement.¹⁹ As stated in a *Lancet* editorial, “the Achilles heel of the [genotypic] technique is interpretability.”²⁰ Interpretation of test results requires an appreciation of the range of mutations selected for by various antiretroviral agents as well as the potential for cross-resistance to other drugs conferred by some of those mutations. This information can be found on the web site at URL: <http://www.hiv-web.lanl.gov>. Consultation with an expert in HIV drug resistance is encouraged to facilitate interpretation of genotypic results.¹⁶

PHENOTYPIC TESTING

Phenotypic testing determines the ability of the patient's pathogen, in this case HIV, to grow when subjected to various medications. The virus is grown in culture using peripheral blood mononuclear cells or similar media, after which it is mixed with differing concentrations of antiretroviral agents. Viral growth is then examined, leading, in turn, to calculations of inhibitory concentrations, such as IC_{50} and IC_{90} . This type of testing is routinely performed for bacteria and other human pathogens.¹⁶

Instead of being subject to the interpretation dilemmas associated with genotypic testing, the results of phenotypic testing are drawn directly from observation of viral growth following exposure to drugs. Accordingly, phenotypic analysis is considered by many to better reflect the true response of HIV to a specific agent. Again, interpretation of test results requires an appreciation of the range of mutations selected for by various antiretroviral agents as well as the potential for cross-resistance to other drugs conferred by some of the mutations and is complicated by the paucity of data on the specific level of resistance associated with drug failure. Consultation with an expert in HIV-drug resistance is encouraged to facilitate interpretation of phenotypic results.¹⁶

PROBLEMS ASSOCIATED WITH BOTH GENOTYPIC AND PHENOTYPIC TECHNIQUES

The most prominent deficiency associated with both modes of testing is lack of comprehensiveness: not all strains within a given patient are examined. Assay techniques test only the most concentrated strains found in serum. Any strain constituting less than 10%–20% of total circulating virus will essentially be missed. In many acute and subacute infections, drug-susceptible wild-type strains vastly predominate, and this domination means that resistant strains found in small concentrations will not contribute to the results of either genotypic or

phenotypic testing. As a result, resistant strains may emerge late in the infection under selective pressure once the nonresistant strains have been treated with a given regimen, even though resistance testing had not originally indicated that the virus may have been resistant to that regimen. Therefore, resistance assays should be performed while the patient is still on antiretroviral agents. Results suggesting the absence of resistance should be interpreted carefully in relation to the prior treatment history.¹⁶

Additional issues include the costs of the procedures and the time required to attain results (days to weeks); these obstacles are considerably more significant for the phenotypic technique. The lack of uniform quality assurance for all assays, as well as the potential for disagreement between the findings of genotypic and phenotypic testing for the same patient, must also be considered.¹⁶

The interpretation of specific genotypic resistance patterns needs to be better defined and clinical cut-off points for phenotypic resistance need to be established.¹⁵

CURRENT UTILITY OF RESISTANCE TESTING

In spite of its limitations, HIV-AIDS practitioners and researchers are successfully applying the technique of resistance testing in patient management. Resistance testing is recommended as a useful tool in selecting active drugs when antiretroviral regimens are being changed in the setting of virologic failure and for suboptimal suppression of viral load after initiation of antiretroviral therapy. Because of potential transmission of resistant strains of HIV, resistance testing may be considered for patients with acute infection. These assays are not recommended for chronic HIV infection before initiation of therapy or after discontinuation of medications or for patients with a plasma viral load of less than 1,000 HIV RNA/ml.¹⁶

There is currently no prospective data to support the use of one type of resistance testing assay over the other (i.e., genotypic versus phenotypic)

in different clinical situations. One type of assay is generally recommended per sample; however, in the setting of complex prior treatment history, both assays may provide important and complementary information. Because of faster turnaround time, the use of a genotypic assay may be preferred for acute HIV.¹⁶ Physicians are encouraged to check with the laboratories of their associated hospitals as well as local commercial laboratories to determine the availability of testing for their own patients.

In general, recommendations for resistance testing in pregnancy should be the same as for non-pregnant patients: acute HIV infection, virologic failure on an antiretroviral regimen, or suboptimal viral load suppression after initiation of antiretroviral therapy are all appropriate indications for resistance testing. Additionally, resistance testing is recommended for pregnant women with a high likelihood of having resistant virus, based on community prevalence of resistant virus, known drug resistance in the woman's sex partner, or other source of infection.²¹ If an HIV positive pregnant woman is taking an antiretroviral regimen that does not include zidovudine, or if zidovudine was discontinued because of maternal drug resistance, intrapartum and neonatal zidovudine prophylaxis should still be administered to prevent mother-to-infant HIV transmission.¹⁶ Although perinatal transmission of resistant virus has been reported, it appears to be unusual, and it is not clear that the presence of mutations increases the risk of transmission.²¹

Although physicians should incorporate resistance testing in the medical management of their patients with HIV disease, the following guidelines should be considered:

- There is no substitute for a thorough treatment history when physicians are considering the components of an antiretroviral treatment regimen.
- No therapeutic decision should be made based on the results of resistance testing alone; virologic failure (as well as patient intolerance, etc.) remains the primary reason for changing regimens, and the entire clinical picture must be considered.
- HIV care is complex and should be supervised by a physician with expertise and extensive experience treating HIV-infected persons.
- Efforts should be aimed at maximizing adherence. *NJM*

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CME EXAMINATION: DEADLINE SEPTEMBER 30, 2004

“Hiv Resistance Testing”

1. Which of the following is a clinical indication for HIV resistance testing?
 - A. After discontinuation of drugs
 - B. Chronic HIV infection prior to initiation of therapy
 - C. Plasma viral load <1000 HIV RNA/ml, as measured by RT-PCR
 - D. Virologic failure during antiretroviral therapy
2. Which of the following is not a clinical indication for HIV-resistance testing?
 - A. Acute HIV infection
 - B. Pregnancy in HIV-infected women
 - C. Suboptimal suppression of viral load after initiation of antiretroviral therapy
 - D. Virologic failure during antiretroviral therapy
3. Which of the following most accurately describes resistance testing?
 - A. Genotypic testing subjects the patient's HIV virus to varying medications
 - B. Genotypic testing defines the patient's genetic structure
 - C. Phenotypic testing defines the patient's HIV genetic structure
 - D. Phenotypic testing subjects the patient's HIV virus to varying medications
4. Which of the following is not a limitation of HIV resistance testing?
 - A. Lack of standardized break points defining resistance for all antiretroviral agents
 - B. Resistance testing cannot be performed when the plasma viral load >1000 HIV RNA/ml, as measured by RT-PCR
 - C. Results of genotypic tests may vary from laboratory to laboratory and require intricate interpretation by an expert
 - D. The tests identify only the predominant viral populations in circulation. Minority species that represent less than 10%–20% of the viral population may be missed
5. The prevalence of resistant strains of HIV is:
 - A. Decreasing
 - B. Increasing
 - C. Unchanged

ANSWER SHEET

“Hiv Resistance Testing”

Darken the correct answers

1. ☐ A ☐ B ☐ C ☐ D2. ☐ A ☐ B ☐ C ☐ D3. ☐ A ☐ B ☐ C ☐ D4. ☐ A ☐ B ☐ C ☐ D5. ☐ A ☐ B ☐ C

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